"On the Presence of Tyrosinases in the Skins of some Pigmented Vertebrates.—Preliminary Note." By FLORENCE M. DURHAM. Communicated by W. BATESON, F.R.S. Received November 10,—Read December 1, 1904.

(From the Balfour Laboratory, Cambridge.)

In the course of an investigation of the "heredity of coat-colour in mice," the necessity for further knowledge, concerning the formation of pigment, became evident.

In the hairs of these animals, there are apparently three pigments (probably different forms of melanins), yellow, chocolate, and black. The characteristic colour of each mouse is due to the presence of one or more of these pigments. Thus, the yellow or "fawn" mouse has only yellow pigment in its hairs, the chocolate mouse has only the chocolate pigment, a black mouse has both chocolate and black pigments, while the house mouse or "agouti" mouse has all three pigments in its hairs (1).

The differences in the nature of these pigments have not been worked out as yet. Much work, however, has been done upon the origin of melanins and on their relationships to the aromatic decomposition products of proteids.

In 1901, v. Fürth and Schneider (2) showed that a tyrosinase could be obtained from the blood of certain insects. This tyrosinase acted upon a chromogen present in the blood, and converted it into a melanin-like substance. When a solution of tyrosin in water was treated with the ferment, a melanin-like body was also obtained. According to the observations of Przibram (which are published with those of v. Fürth and Schneider), a tyrosinase can be extracted from the ink-sac of *Sepia officinalis*. This ferment, acting in a like manner upon a watery solution of tyrosin, yields a black pigment, probably a melanin. Ducceschi (3) has shown that a similar ferment can be obtained from the blood of *Bombyx mori*.

Arguing from these facts, Cuénot (4), in a paper on the "Heredity of Coat-Colour in Mice," suggests that the formation of pigment in these animals is due to the presence, either of different chromogens and one ferment, or of two different ferments and one chromogen. The results of the action of the ferment on the chromogen would be to produce the characteristic colour of the mouse. The alteration of colour would be due either to the presence of a different chromogen, or to the presence of a different ferment.

It seemed worth while, therefore, to determine whether a ferment could be extracted from animals, which, like the mouse, are deeply pigmented in their coats; and to test whether, if such a ferment could

be obtained from the skins of these animals, pigmented substances corresponding in colour to those of the animals taken, could be formed by the action of the ferment upon tyrosin or allied bodies.

I chose, for this purpose, the skins of young or fœtal animals, because these are in a condition of active pigment-formation. The study of adult forms shows that the older animals moult gradually, and that they would, therefore, in all probability, yield less material.

As young mice are very small, I preferred to use new-born rabbits and rats (of black or agouti origin) and fœtal guinea-pigs (about 8 weeks). In the case of the guinea-pigs, the skins of the mothers were also used for comparison.

The skins, having been removed from the animals, were chopped in a sausage-machine, and ground in an agate mortar with kieselguhr and distilled water. The use of this mortar was kindly permitted to me by Professor Sims Woodhead. The ground-up skins were then pressed out as completely as possible.

The expressed juice was red, and opaque in appearance. A portion of it was placed in a test-tube with solid tyrosin, and a milligram of ferrous sulphate was added as an activator. Toluol was then added as an antiseptic, and the tube was closed with cotton-wool, and placed in an incubator at 37° C.

For comparison, a series of tubes were prepared, in order to control the experiment. In the first tube boiled juice was placed, to which tyrosin and ferrous sulphate were added. In the second tube juice only was placed. The third tube contained juice, to which tyrosin was added. The fourth tube contained the juice, with a milligram of ferrous sulphate. The fifth tube contained no juice, but tyrosin in distilled water, with ferrous sulphate. These tubes were all placed in the incubator.

A similar series of tubes was also prepared, and kept at the ordinary temperature of the laboratory. A tube containing juice, tyrosin, and ferrous sulphate was also prepared, and kept at the laboratory temperature. In every case, toluol was used as an antiseptic. Cotton-wool was used to close the tubes, on account of the action of iron upon cork, which might introduce an error into the result.

In 24 hours, the contents of the tube, which was incubated, containing the juice, tyrosin, and ferrous sulphate showed a change in appearance. The fluid had darkened and a black substance was deposited; the amount of this substance increased as time went on. In no case did any of the other tubes arranged to control the experiment show any such change.

In one case, when considerable juice had been obtained from a number of young black rabbits, about 2 days old, a certain quantity of the juice was kept, in order to test the effect of time upon it. After it had been tested, it was kept standing for two days and fresh tubes

were prepared from it. The blackening and formation of black substance occurred as before, but 48 hours in the incubator were required before the action commenced. The same juice was found to have lost its activity when kept for a week.

Experiments were then made with extracts of skins, which had been kept in alcohol (95 per cent.). The skins were chopped and ground with kieselguhr and distilled water, and pressed out as before. The resulting extract was colourless and cloudy in appearance. When treated with tyrosin and ferrous sulphate, and incubated, the same results were obtained, viz., darkening of the fluid and the formation of a black deposit. The time of action was delayed, so that about 10 days were necessary for the change of colour to appear.

I also tried the skins of black unhatched chickens, which had been kept in 95 per cent. alcohol, and obtained like results from them. The extract from the skins of the chickens was clear and colourless. After the darkening had commenced, I filtered the solution and added more tyrosin, and in this way, I was able to get a further deposit of black substance.

Solutions were prepared in another way. Juice, which had been obtained from the skins of fresh animals was saturated with ammonium sulphate. The resulting precipitate was filtered off, and was then washed with a solution of saturated ammonium sulphate, dissolved in distilled water and re-precipitated by saturation with ammonium sulphate. On re-dissolving this precipitate in distilled water, a certain portion of it was found to be insoluble. This was filtered off and the filtrate was dialysed against distilled water. When free from sulphate (as shown by testing with lead acetate solution), the clear, colourless fluid was treated with tyrosin and ferrous sulphate and incubated.

As before, pigment was formed, and as in the case of the skins preserved in alcohol, the action was delayed. About 10 days were required.

Amongst other animals tested in this way, I used self-coloured "red" guinea-pigs. I used the fœtal young and also the mother for comparison. The hair of this kind of guinea-pig contains only one pigment, deep yellow or orange in colour.

In the case of the young guinea-pigs, the solution became yellow and an orange-coloured deposit was formed. I filtered this off, and on the addition of more tyrosin, a fresh orange deposit was obtained. The extract made from the skin of the mother also changed in colour and yielded a yellow deposit, but less in amount than in the case of the young animals.

Hitherto, material from white or albino animals has yielded no results, but the animals obtained were too few for final conclusions to be formed in regard to them. Up to the present, only minute quantities of the coloured substances have been obtained, insufficient for complete analysis. They resemble melanins in being soluble in alkalis and

insoluble in mineral acids. Further experiments are in progress, on a larger scale, to endeavour to produce enough for a thorough analysis.

Summary.

An extract can be made from the skins of certain pigmented animals (rabbits, rats, guinea-pigs and chickens), which will act upon tyrosin and produce a pigmented substance. This action suggests the presence of a tyrosinase in the skins of these animals.

The action of the tyrosinase is destroyed by boiling, does not take place in the cold, is delayed by time, requires a temperature of about 37° C., and also the presence of an activating substance such as ferrous sulphate to start it.

The coloured substances produced are in accordance with the colour of the animals used. Black substances are obtained, when animals with black pigment in their skins are used, and yellow substance, when the skin contains the orange pigment. The coloured substances are soluble in alkalis, but insoluble in acids.

In conclusion, I take this opportunity to express my gratitude to Dr. Hopkins, to whom I am indebted for help and advice in the foregoing experiments.

LITERATURE.

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(4) Cuénot. 'Arch. d. Zool. Expér. et Gén., Notes et Revue,' vol. 1.